B2 cont

The use of commercially available nucleotide analogs conjugated to fluorescein is of particular value for clinical applications since it circumvents time-consuming and sometime troublesome immunological steps required to visualize haptenized probes. In addition, this results in an improved signal/noise ratio, which could enhance overall detection sensitivity, especially if a cooled CCD camera were used for imaging. It can be expected that other nucleotides with additional conjugated fluorophores will be available soon, which would both simplify and expand the combinatorial labeling strategy for multicolor hybridization assays even more.---

In the Claims

Cancel claims 1-3, 5-7, 9-10 and 12-16.

Add the following new claims 19-35:

B3

19. A method of DNA hybridization for detecting target chromosomal DNA in situ in interphase cells, comprising:
    a) providing labeled probe DNA having sequences specifically hybridizable to target chromosomal DNA, but having repetitive sequences which cross-hybridize to non-target chromosomal DNA, and competitor DNA containing the repetitive sequence, the labeled probe DNA and the competitor DNA comprising fragments less than about 500 nucleotides in length;
    b) combining
        i) the labeled probe DNA;
        ii) the competitor DNA; and

B3 cont.

iii) a sample of interphase cells, or nuclei thereof, treated to render chromosomal DNA therein available for hybridization with the probe DNA,

under hybridization conditions wherein cross-hybridization between repetitive sequences in the probe DNA and the sample is sufficiently suppressed to allow the probe DNA to hybridize essentially specifically to the target chromosomal DNA; and

c) detecting the labeled probe DNA in order to detect the target chromosomal DNA.

20. The method of claim 19, wherein the labeled probe DNA and the competitor DNA are combined before combination with the sample of interphase cells or nuclei thereof.

21. The method of claim 20, wherein the labeled probe DNA and the competitor DNA are combined under conditions which allow the repetitive sequences in the competitor DNA and repetitive sequences in the labeled probe DNA to preanneal and to thereby suppress cross-hybridizing repetitive sequences sufficiently to produce labeled probe DNA hybridizable essentially specifically to the target chromosomal DNA.

22. The method of claim 19, wherein the interphase cells are tumor cells or nuclei thereof.

23. The method of claim 19, wherein the interphase cells comprise uncultured cells from amniotic fluid, or nuclei thereof.

24. The method of claim 19, wherein the target chromosomal DNA is an individual chromosome or a region thereof.

B3cont

25. The method of claim 24, wherein the labeled probe DNA is DNA obtained from a library of DNA of the target chromosome.

26. The method of claim 19, wherein the labeled probe DNA and the competitor DNA comprise DNA fragments ranging from 150-250 nucleotides in length.

27. The method of claim 19, wherein the competitor DNA for suppression of repetitive sequences is total human genomic DNA.

28. The method of claim 19, wherein a carrier DNA is added to the combination of labeled probe DNA, competitor DNA and the sample preparation.

29. A method of claim 21, for simultaneous detection of more than one chromosome, or region thereof, wherein probe DNA for each chromosome, or region thereof, is labeled with a different fluorophor or a different combination of fluorophors to yield an optically distinguishable signal.

B3cont

30. A method of <u>in situ</u> hybridization for distinguishably labeling individual human chromosomes, or regions thereof, comprising

a) providing labeled probe DNA for each chromosome, or region thereof, to be visualized, each probe DNA having sequences specifically hybridizable to target chromosomal DNA, but having repetitive sequences which cross-hybridize to non-target chromosomal DNA and each probe DNA being labeled with a different fluorophor or a different combination of fluorophors to generate optically distinguishable signals; and

b) combining

i) the labeled probe DNA for each chromosome, or region thereof;

ii) a competitor DNA containing the repetitive sequences; and

iii) a sample of cells, or nuclei thereof, treated to render chromosomal DNA therein available for hybridization with the labeled probe DNA,

under hybridization conditions wherein cross-hybridization between repetitive sequences in the probe DNA and the sample is sufficiently suppressed to allow each probe DNA to hybridize essentially specifically to the target chromosomal DNA, to thereby distinguishably label individual chromosomes.

B3 cont.

31. A method of <u>in situ</u> hybridization for visualizing an individual human chromosome, or region thereof, comprising

a) providing biotinylated DNA fragments smaller than 500 nucleotides derived from a DNA library of a target chromosome, or region thereof, for visualization;

b) combining the biotin-labeled DNA fragments with fragments of total genomic DNA having the same size distribution as the labeled fragments and incubating the mixture under initial conditions that allow denaturation of the fragments and, subsequently, under conditions that promote annealing of fragments containing repetitive sequences but not fragments containing chromosome-specific sequences, to produce probe DNA essentially specific for the target chromosome;

c) combining the probe DNA with a cellular preparation, treated to render target chromosomal DNA available for hybridization with the probe DNA, under conditions which allow the probe DNA to hybridize to the target chromosomal DNA; and

d) detecting the probe DNA by incubating the preparation with an avidin conjugated fluorophor and the detection of the fluorescent signal.

B3 cont.

32. A method of <u>in situ</u> hybridization for simultaneously visualizing individual human chromosomes, or regions thereof, comprising

a) providing labeled probe DNA for each chromosome, or region thereof, to be visualized, each probe DNA having sequences specifically hybridizable to target chromosomal DNA, but having repetitive sequences which cross-hybridize to non-target chromosomal DNA and each probe DNA being labeled with a different fluorophor or a different combination of fluorophors to generate optically distinguishable signals;

b) combining

i) the labeled probe DNA for each chromosome, or region thereof;

ii) a competitor DNA containing the repetitive sequences; and

iii) a sample of cells, or nuclei thereof, treated to render chromosomal DNA therein available for hybridization with the labeled probe DNA,

under hybridization conditions wherein cross-hybridization between repetitive sequences in the probe DNA and the sample is sufficiently suppressed to allow each probe DNA to hybridize essentially specifically to the target chromosomal DNA; and

c) detecting the optically distinguishable signals generated by each probe DNA to visualize each chromosome.

B Cont.

33. A method according to claim 32, wherein the detecting step includes the steps of

a) generating one or more digital images of the hybridized chromosomal DNA; and

b) visually emphasizing those portions of the digital images which represent optically distinguishable signals associated with a fluorophor or combination thereof.

34. A method according to claim 33, wherein the generating step includes the step of producing each the digital image by imaging the hybridized chromosomal DNA with a filter having a bandpass corresponding to each of a respective one of the fluorophors.

35. A method according to claim 34, wherein the visually emphasizing step includes the steps generating an image of the hybridized chromosomal DNA by

a) assigning a respective color to each fluorophor; and

b) substituting, for those portions of the digital images which represent optically distinguishable signals associated with the fluorophors, or combinations thereof, respective colors assigned thereto.

Remarks

This amendment is submitted as part of a file-wrapper-continuation in part of Application Serial No. 07/271,609 filed November 15, 1988. Figure 15 and a new Example III have been added. All claims have been replaced by newly written claims 19-35.

The new claims define the invention more particularly and comply with the requirements of §112. Support for the new claims can be found throughout the specification and in the claims as originally filed. Specifically, support for claim 19 appears, inter alia, at pages 14-19 and at pages 39-52. Sources of the labeled probe DNA are described at page 15, lines 3-28. The labeling of the DNA is described at page 15, line 29 and page 16, line 12. The preannealing step of claims 21 and 30 is described at page 17, line 27 - page 18, line 26 and in further detail at pages 35-36 and at pages 39-52. Application of the method to interphase cells such as tumor cells (claim 22) and uncultured interphase cells from amniotic fluid (claim 23) is described, inter alia, at page 20, lines 3-9 and page 29, line 15. Support for the fragment size distributions recited in claims 19, 26 and 30 is found at page 16, lines 15-18. Support for the use of total genomic DNA as competitor DNA (claim 27) is found at page 17, lines 7-11. Carrier DNA (claim 28) is described at page 18, lines 14-22. Combinational labeling for detection of more than one chromosome (claims 29-34) is described at pages 23-25 and in the material added by amendment.

Applicants' invention is a method of in situ hybridization for detecting chromosomal DNA in interphase nuclei. The method can be used to label entire individual human chromosomes, or regions thereof, or to visualize a plurality of different chromosomes simultaneously in order to evaluate numerical and structural aberrations of chromosomes (for example, chromosomal

aneuploidy and chromosomal deletions, rearrangements and translocations). In Applicants' method, the hybridization probe can be made from libraries of DNA from individual chromosomes. Such libraries are readily available for all human chromosomes. The DNA from these libraries contains sequences specific to a chromosome but it also contains sequences which are non-specific such as the ubiquitous repetitive sequences Alu and KpnI which appear in all chromosomal DNA. Thus, although this DNA will hybridize to the target chromosome, it will also cross-hybridize to other chromosomes, prohibiting specific detection of the target chromosome.

According to the method of this invention, probe DNA fragments of less than 500 nucleotides are used. The cross-hybridization of probe repetitive sequences to cognate repetitive sequences in non-targeted chromosomes can be suppressed by a competitor DNA containing the repetitive sequences. For example, DNA derived from a single chromosome library can be suppressed with total human genomic DNA to provide chromosome-specific hybridization. In one embodiment, the probe DNA and competitor DNA are preannealed to suppress cross-hybridizing sequences. The probe DNA is then hybridized to cellular or chromosomal preparation to specifically label a target chromosome, or a region of the chromosome. The label associated with the probe is detected to visualize the individual chromosome, or region thereof.

By combinational labeling, probe sets specific for different chromosomes can be distinguished, allowing for simultaneous visualization of more than one chromosome in a sample. Probe can be labeled different fluorophors and different combinations of fluorophors to generate optically distinguishable signals for visualization of individual chromosomes.

This method of in situ suppression hybridization permits rapid analysis of samples and decreases the time to make diagnosis of genetic disorders such as Down's Syndrome. The procedure can be without the need to culture cells (e.g., fetal cells obtained by amniocentesis) or to obtain metaphase spreads (e.g., interphase tumor cells).

The Examiner objected to the specification and rejected all claims under §112, first paragraph. This objection is obviated by amendment of the claims. As pointed out above, the new claims are fully supported by the specification.

All claims were rejected under §103 as being unpatentable over SU 1203108 in view of Singer et al. and Dattagupta et al. This rejection is obviated by amendment of the claims. SU 1203108 teaches a method detecting human chromosome 11 using a probe specific for the pre-central region of chromosome 11. This is an example of one probe which is specific for a particular chromosome. In Applicants' method, probes are fragments less than 500 nucleotides which contain repeat and specific sequences and they are made specific by suppressing non-specific sequences. Such probes can be made for any chromosome from, for example, libraries of genomic chromosomal DNA. Singer describes a method of in situ hybridization and Dattagupta teaches a method for labeling nucleic acids. None of these references teaches or suggests suppression of repetitive sequences in a probe DNA by a competitor DNA for specific hybridization in interphase cells. Further, these references do not teach or suggest combinational labeling techniques for visualizing more than one chromosome simultaneously by in situ hybridization.

All claims were rejected under §102(a) as being clearly anticipated by Cremer et al. and [sic; or?] Pinkel et al. This rejection is obviated by amendment of the claims.

Cremer et al. teach that two repetitive DNA sequences which are confined to chromosomes 1 and 18 can be detected with labeled hybridization probes. Thus, the probes can be used to detect these particular chromosomes. Some cross-hybridization of the probe for chromosome 18 (probe 18c) was observed. There is no teaching of the claimed hybridization assay for specifically detecting chromosomes in interphase cells or the labeling technique to visualize a plurality of chromosomes.

Pinkel et al. teach a method of staining human chromosomes in interphase nuclei by in situ hybridization. According to their method, "labeled probe DNA is mixed with unlabeled human genomic DNA, denatured and incubated before applying it to the denatured target cells". The authors do not teach or suggest suppression of repetitive sequences in the probe DNA. As in Cremer et al., there is no teaching or suggestion of the claimed method of combinatorial labeling for visualizing more than one chromosome simultaneously in a sample.

All claims were rejected under §102(b) as being clearly anticipated by Gray et al. and [sic; or ?] Landegent et al. This rejection is obviated by amendment of the claims.

Gray et al. teach fluorescence hybridization in metaphase spreads to human chromosome 21 using a composite collection of biotin-labeled probes from a library made from DNA isolated from human 21 chromosomes. In one approach to eliminate non-specific hybridization, unlabeled whole genomic human DNA was used for prehybridization and during hybridization to block binding of non-specific repeat sequences in the biotin-labeled probe DNA. Applicants' method can be used to label chromosomes for interphase cells. Further, Applicants' method of combinational labeling can be used to visualize more than one chromosome in the same sample.

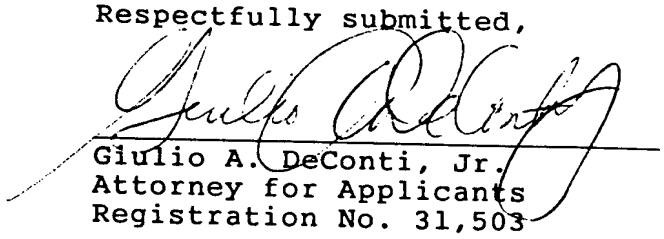
Landegent et al. describe an in situ hybridization technique for chromosomal localization in which the presence of highly repetitive sequences is eliminated with competition hybridization with Cot-1 DNA. As in Gray et al., the work described was performed in metaphase spreads. There is no teaching or suggestion of whether or how in situ hybridization can be performed in interphase cells to result in chromosome specific labeling. Further, there is no teaching or suggestion of the claimed method of combinatorial labeling for visualizing more than one chromosome simultaneously in a sample.

All claims were rejected under §102(f). The basis for this rejection is uncertain because the Examiner has not cited a reference or provided other evidence supporting the contention that the claimed invention was not made by Applicants. The Examiner states that the rejection is based on the Cremer et al. reference. If the Examiner means the same Cremer et al. reference cited in support of the rejection under §102(a), then Applicants' comments directed to that rejection apply to the rejection under 102(f) and it should be withdrawn.

Conclusion

The claims are now in condition for allowance and Applicants respectfully request early indication of such. If the Examiner feels that a telephone conversation will expedite prosecution of the application, she is invited to call the undersigned at (617) 227-7400.

Respectfully submitted,



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